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CITATION:

KAMITSUJI, Hisatoshi ...[et al]. <Preliminary>Studies on the Production of Manganese Peroxidase by a White-rot Fungus Pleurotus ostreatus. Wood research : bulletin of the Wood Research Institute Kyoto University 1999, 86: 41-42

ISSUE DATE:

1999-09-30

URL:

http://hdl.handle.net/2433/53161

Preliminary

Studies on the Production of Manganese Peroxidase by a White-rot Fungus *Pleurotus ostreatus*

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(Received May 31, 1999)

Keywords: Manganese peroxidase, isoenzyme, white-rot fungi, *Pleurotus ostreatus*

Manganese peroxidase (MnP) was secreted by *P. ostreatus* in liquid stationary culture. Two different MnP isozymes were secreted in glucose/yeast-extract medium (GY) and peptone/glucose/yeast-extract medium (PGY). The isoelectric points of MnP produced in GY medium (MnP-GY) and PGY medium (MnP-PGY) were found to be 3.70 and 3.95, respectively. The molecular masses of both isozymes were the same 42 kDa. MnP-PGY found to be the same N-terminal sequences of MnP 3 (data not shown). On the other hand MnP-GY was detected only in the solid culture[2].

1. Production of manganese peroxidase by *P. ostreatus*

P. ostreatus (ATCC 66376) was cultivated in a glucose-peptone-yeast-extract medium (PGY) and glucose-yeast-extract medium (GY) stationarily at 28°C under darkness. A time course study was performed to compare the production of MnP by *P. ostreatus* in GY and PGY medium. As shown Fig. 1, maximum MnP activities were detected in 13 day-old PGY culture and 30 day-old GY culture. The activities of MnP produced in PGY (MnP-PGY) and that in GY (MnP-GY) were 0.5 and 1.6 U/ml, respectively and the specific activities were 6 and 128 U/mg, respectively. MnP-GY was produced from 10 and 30 days as mycelium grew, suggesting MnP-GY production is likely primary metabolite. On the contrary, however, in PGY the maximal activity was found after the maximal growth was achieved.

2. Purification of MnP from *P. ostreatus*

The culture filtrate was dialyzed against 20mM Na-succinate buffer (pH 4.5). The dialyzate was concentrated by ultrafiltration (Amicon PM-10) and then applied to a Sepharose CL-6B column as shown in Fig. 2. Fractions which showed MnP activity were pooled and concentrated by ultrafiltration. The concentrate was subjected to ion-exchange chromatography on a Pharmacia Mono-Q column (10/10). The elution was carried out with Na-succinate buffer (pH 4.5) using NaCl gradient of 0 to 100 mM at a flow rate of 1.0 ml/min. The fractions showing MnP activity were pooled and concentrated to 1 ml through a Centriprep-30 microconcentrater (Amicon). Specific activities were 269 and 324 U/mg for MnP-GY and MnP-PGY, respectively. Enzyme purity was confirmed by SDS-PAGE using a FastGel gradient 10–15

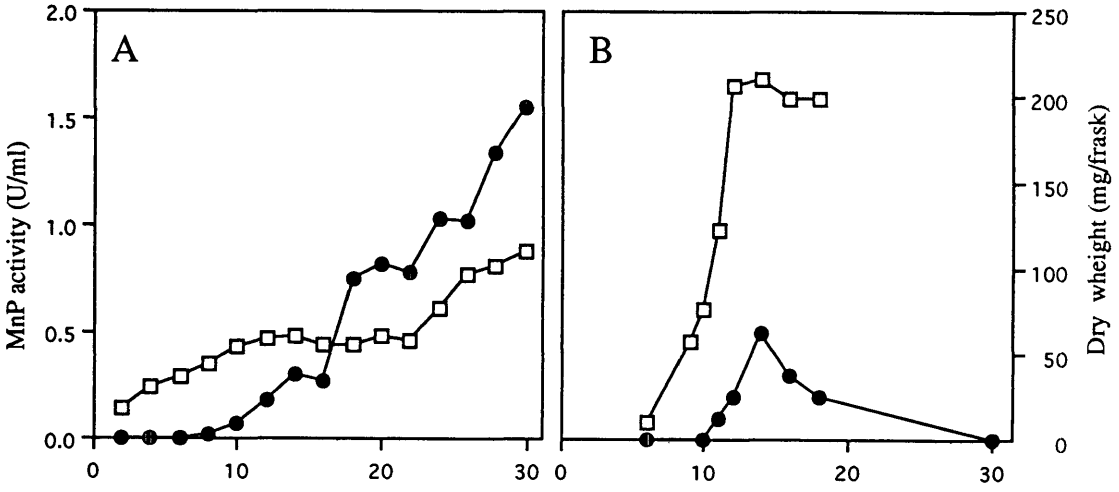

Fig. 1. Production of MnP by *P. ostreatus*. (A), GY medium (B), PGY medium
MnP activity, (—●—); Dry wheight, (—□—).

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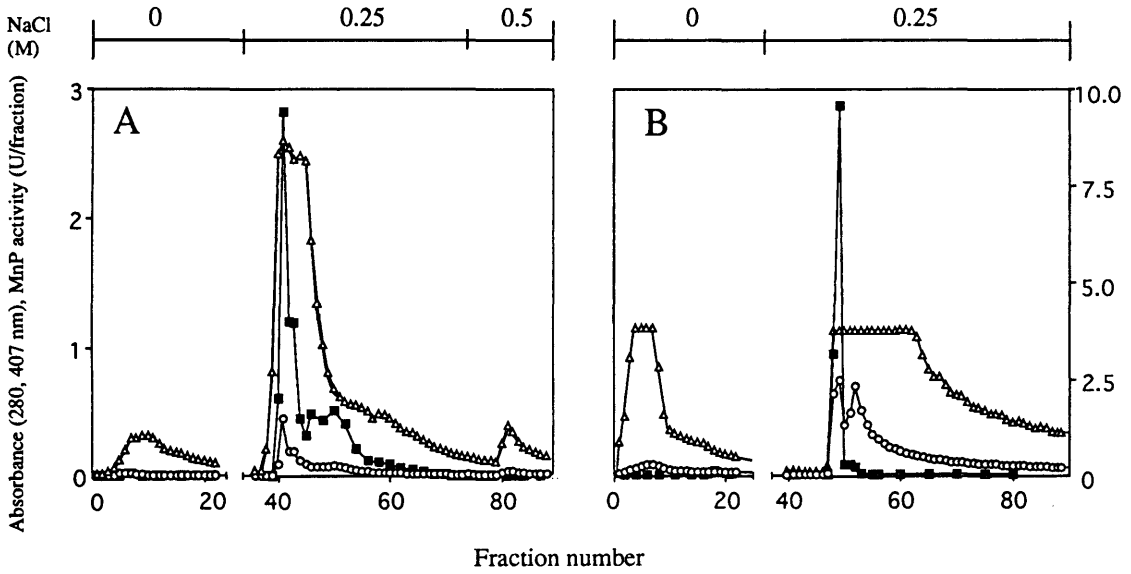

Fig. 2. Separetion of MnP from *P. ostreatus* by DEAE. Sepharose chromatography. (A), GY medium. (B), PGY medium. MnP activity was determined using guaiacol as a substrate. Profiles corresponding to MnP (---■---) activities, absorbances at 280 nm (···△···) and 407 nm (---○---) are shown.

(Pharmacia) and isoelectroforcusing (IEF) analysis using a Servalyt 2–4 (Pharmacia). N-terminal sequences of *P. ostreatus* MnP-GY and MnP-PGY were determined, as follows,

MnP-GY : VTCATGQTTANE
MnP-PGY : ATCADGRTTANA

The N-terminal sequences of MnPs showed high similarity with that of other strains of *P. ostreatus*[1,2] as well as that MnP isolated from *P. eryngii* and *P. pulmonarius* However, the sequences were found to be different from those of MnPs from *Phanerochaete chrysosporium* and *Lentinus edodes*. The N-terminal sequence of MnP-GY was also found in the MnP produced in the sawdust/H_2O medium but not in liquid culture.

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